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Thomas G. Fanning Oct. 18, 1995
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Table of Contents

1. Front Cover.....	1
2. SF 298.....	2
3. Foreword.....	3
4. Table of Contents.....	4
5. Introduction.....	5
6. Experimental.....	5
7. Conclusions.....	6
8. References.....	6

Introduction

Human *LINE-1* (*L1Hs*) is a transposable element that encodes a reverse transcriptase and moves *via* an RNA intermediate [1]. It therefore seems possible that cells in which *L1Hs* is active may be subject to insertional mutagenesis. We have recently found that the element is expressed in a significant number of germ cell cancers [2,3] as well as in many breast tumors and breast tumor cell lines [4]. This last finding raises the possibility that the initiation or progression of some breast cancers is facilitated by *L1Hs*-induced insertional mutations.

In addition to insertional mutagenesis, there are several other characteristics of the *L1Hs* element that suggest its potential as an oncogenic agent. For example, *L1Hs* has an internal promoter which could potentially lead to readthrough transcription and activation of downstream genes. In addition, the *L1Hs*-encoded p40 protein has a leucine zipper motif, suggesting a possible interaction with other cellular proteins. Such interactions at inappropriate times might lead to the disruption of important cellular functions. Thus, *L1Hs* involvement in cancer could occur by several mechanisms, either singly or in combination.

Our long range goals focus on the isolation of cellular genes that are affected by *L1Hs* transposition. These genes are presumably the ones whose inactivation (by insertional mutagenesis), or activation (by readthrough transcription), is one of the steps in the pathway leading to malignancy. Specifically we have proposed to:

- (1) Place a "tagged", transpositionally competent *L1Hs* element into non-malignant human breast epithelial cells.
- (2) Identify malignant cells arising from the non-malignant cell population and isolate and characterize sequences into which the tagged *L1Hs* element has transposed.

Experimental

In our initial experiments we have used vectors containing only the first *L1Hs* open reading frame (p40 gene). We have done this for several reasons; (1) we have an anti-p40 antibody that is capable of detecting small quantities of the protein. Thus, we can easily assay cells for *L1Hs* expression. (2) Experience gained in this work will be very useful in placing the full-length *L1Hs* element into the same cells.

Transfection of breast epithelial cells.

We have used three human, non-malignant, breast epithelial lines in our work: HBL100, Hs587Bst, and MCF10A. Transfection protocols have utilized (a) calcium phosphate-DNA coprecipitation, (b) a DEAE-dextran-DNA mix, and (c) several liposome-DNA complexes (LipofectAMINE, LipofectACE, and Lipofectin, all sold by Gibco-BRL). Our initial experiments utilized a β -galactosidase-containing vector, since measurement of the enzyme activity in cell extracts is very sensitive. Using this vector, the HBL100 cells were completely refractory to transfection. In addition, colleagues have informed us that this cell line is refractory to transfection by electroporation. Similarly, we were unable to obtain transfected Hs587Bst cells. Thus, we have concentrated our efforts on the MCF10A cell line.

Transfection of MCF10A cells with the β -galactosidase-containing vector suggested that transfection was possible, but the efficiency was low. Indeed, when we stained a population of transfected cells, it was apparent that less than 1% were actually taking-up DNA under our most optimal conditions. Nevertheless, this is certainly sufficient to give us stably

transfected cells that express *LIHs*, which is our goal. To obtain stably transfected cells we treat with a LipofectACE-DNA complex, then subject the cells, after an appropriate time interval, to a neomycin analog (G418) since the vector contains both the *LIHs* p40 gene and a neomycin (neo) resistance gene. Neo resistant cells are cloned, grown in large numbers, and assayed for p40 expression.

Expression of the *LIHs* p40 gene in transfected cells.

We have placed the following plasmid-based constructs into the MCF10A cell line: RSV-p40, CMV-p40 and MMTV-p40. These vectors place the *LIHs* ORF1 gene (encoding the p40 protein) behind the Rous sarcoma virus promoter/enhancer, the cytomegalovirus promoter/enhancer and the mouse mammary tumor virus promoter/enhancer, respectively. All constructs were initially tested for transient expression in COS cells, an SV40-transformed monkey kidney cell line that is easily and efficiently transfected. All constructs proved to be capable of expressing the p40 protein as shown by Western blotting extracts from the transfected COS cells.

All of the constructs produced stable, drug resistant colonies after transfection into MCF10A cells. However, in no case was p40 detected. We assume, but have not yet shown, that the promoter and/or enhancer driving the expression of the p40 gene is inactivated, probably by methylation. The promoter/enhancer for the neo gene, which is under strong selection, is obviously still functional.

Currently, we are repeating the transfection experiments with the p40 gene in several retroviral vectors: LXSN, LNSX and Elneo [5,6]. The LXSN and LNSX vectors have two promoters, one for the neo gene and one for the p40 gene. Thus, there is the possibility that they too could exhibit drug resistance, but have the p40 promoter/enhancer inactivated. The Elneo vector, however, contains only a single promoter/enhancer. With this vector the neo and p40 genes can both be expressed due to mRNA splicing. Thus, complete transcripts express the p40 protein which is located at the 5' end of the mRNA, while spliced mRNAs express the neo gene product.

Conclusions

A successful execution of the goals of the grant require that a full-length, transpositionally competent *LIHs* element be placed into a non-malignant breast epithelial cell. To date we have been unable to achieve this goal. Either the cell lines we wish to use are refractory to transfection, or the constructs we have used are rendered nonfunctional following transfection. We are currently pursuing other vectors that may offer a higher possibility of success. In addition, we are searching for other cell lines that may be more amenable than those we are currently using.

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